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SYNTHESIS AND STUDY OF THE PROPERTIES OF HYBRID COLLAGEN–POLYACRYLAMIDE HYDROGELS

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Hybrid collagen–polyacrylamide hydrogels were synthesized and their properties were investigated using copolymers of acrylamide, acrylic acid, and N,N'-methylenebisacrylamide in the presence of a polymeric structuring agent. The three-dimensional polymer network was formed by polycondensation in an acidic medium at elevated temperature. The effect of collagen content on the structure, crosslinking degree, mechanical properties, swelling behavior, and collagen release kinetics of the hydrogels was studied. The incorporation of collagen into the hydrogel matrix was shown to decrease the gel fraction and Young's modulus because of a reduced effective crosslink density and the formation of a heterogeneous fibrillar structure. A nonmonotonic dependence of the swelling degree on collagen concentration was observed, which was attributed to the competition between osmotic effects and topological constraints of the polymer network. Investigation of collagen release at physiological temperature (36^0C) revealed a two-stage process and a pronounced dependence on the release medium: up to 90% of collagen was released in water, whereas up to 75% was released in model exudate. The obtained results demonstrate the potential of collagen–polyacrylamide hydrogels as a basis for the development of wound dressings with controlled mechanical properties and prolonged release of biologically active components.

Keywords: polyacrylamide, collagen, hydrogel, swelling, release kinetics, wound dressings.

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Introduction

Polymeric hydrogel composites are widely used in many fields, including medicine and biomedicine, wastewater treatment, and agriculture. They serve as a basis for drug delivery systems [1,2], wound and burn dressings [3], personal hygiene products [4], fertilizers with prolonged release of active substances [5], soil additives for water retention [6], cosmetics, and sorbents for wastewater purification [7]. In contrast to single-component hydrogels (in terms of polymer chain composition), composite hydrogels acquire a set of new properties due to the synergistic effects of their constituents and are therefore more versatile in practical applications [8,9].

Depending on the nature of the gel-forming components, composite hydrogels are classified as natural, synthetic, or hybrid. It is evident that natural composite hydrogels are generally preferred because

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natural polymers, gel-forming agents, and products based on them possess biocompatibility, biodegradability, and are obtained from renewable sources, among other advantages [10]. Alginate, collagen and gelatin, pectin, starch, chitosan, cellulose, and hyaluronic acid are widely used in the design of composite hydrogels for various applications [11,12]. The use of hydrogels derived from synthetic raw materials is limited primarily by sustainability and environmental safety considerations, since many of them generate toxic substances during biodegradation. However, a number of synthetic polymers are used in hydrogel production, including those based on polyhydroxy acids, such as polyethylene glycol, poly(glycolide-co-lactide), poly(vinyl alcohol), poly(*N*-isopropylacrylamide), polyacrylamide, poly(*l*-lactic acid), and poly(*l*-lactide). Hydrogel materials obtained from these polymers ultimately degrade into the corresponding hydroxy acids, i.e., harmless degradation products, via hydrolytic cleavage of their ether bonds [13].

The development of hybrid hydrogels (composites), in which component integration occurs at the nanometer or molecular level, is advancing rapidly. Such systems often combine organic and inorganic materials or natural and synthetic polymers to achieve synergistic properties [14]. This approach makes it possible to overcome certain limitations of conventional polymeric hydrogels and enables unprecedented combinations of properties, which is of particular importance for modern high-tech hydrogel applications, especially in medicine.

The aim of this study was to synthesize and investigate the properties of hybrid hydrogels based on collagen and copolymers of acrylamide, acrylic acid, and *N,N'*-methylenebisacrylamide.

Materials and methods

Reagents

Acrylamide (AAm), acrylic acid (AAc), *N,N'*-methylenebisacrylamide (MBAA), collagen, and formaldehyde were supplied by Aldrich and used without additional purification.

Preparation of copolymers based on acrylamide, acrylic acid, and N,N'-methylenebisacrylamide

The copolymers were prepared by free-radical copolymerization in aqueous medium. Ammonium persulfate was used as the initiator, and thioglycolic acid served as the polymer chain growth regulator (Fig. 1).

Acrylamide (20%), acrylic acid (10%), and *N,N'*-methylenebisacrylamide (0.2%) solutions were prepared in water, after which aqueous solutions of thioglycolic acid and ammonium persulfate were added in the required proportions. The reaction mixture was loaded into a jacketed reactor equipped with a stirrer. To remove oxygen, argon was bubbled through the reaction mixture for 20–30 min under continuous stirring. The reactor jacket was then supplied with a heat-transfer fluid at 50^{0}C, and this temperature was maintained in the reaction mixture for 4 h. The resulting copolymer was isolated by precipitation in methanol (mass ratio of methanol to reaction mixture, 4:1). To remove residual unreacted substances, the copolymers were reprecipitated twice. The obtained copolymers were dried to constant weight in a drying oven at 60^{0}C. The synthesis conditions and characteristics of the copolymers are listed in Table 1.

Preparation of the structuring agent based on the obtained copolymers

In a three-necked reactor equipped with a mechanical stirrer and a water bath with controlled temperature, a 20% aqueous solution of the corresponding copolymer was prepared, after which 0.4 N NaOH was added to adjust the pH to 8–10. The solution was heated to 50^{0}C, and the calculated amount of 30% formaldehyde solution was added portionwise in 1 mL aliquots under continuous stirring (Fig. 2). The pH was monitored every 15 min and, when necessary, adjusted back to 8–10 with alkali solution. After the entire calculated amount of formaldehyde had been added, the mixture was stirred for 3 h at 50^{0}C. The pH of the resulting solution was then adjusted to neutral with 5N sulfuric acid.

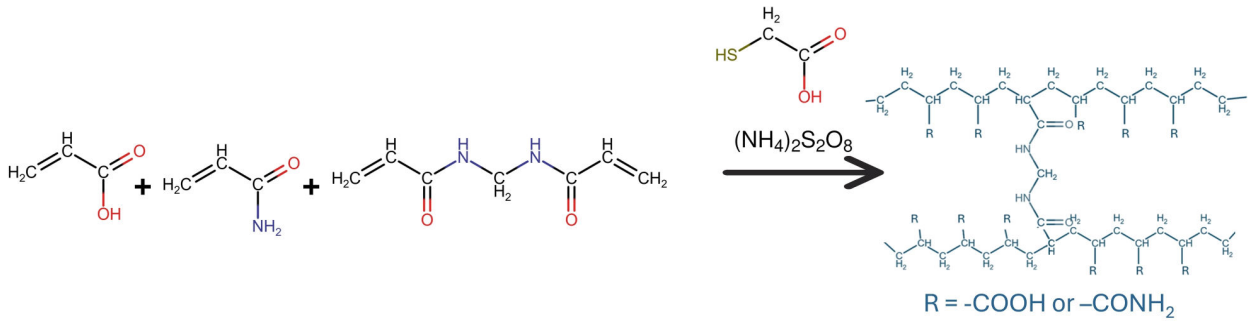


Fig. 1. Scheme of preparation of copolymers based on acrylamide, acrylic acid, and *N,N'*-methylenebisacrylamide

The resulting reactive copolymer (structuring agent) was stored as an aqueous solution with a concentration of 8–25% at +4°C. The copolymer composition and the degree of conversion of amide groups in the copolymer were monitored by NMR spectroscopy in deuterated water.

Preparation of hydrogels based on collagen and acrylic copolymers

Hybrid hydrogels were prepared by copolycondensation in water by mixing solutions of the copolymer, structuring agent, and collagen in the required ratios. The mixture was stirred and its pH was adjusted to 2 with sulfuric acid solution, after which it was poured into polytetrafluoroethylene molds measuring 100×100×4 mm. To carry out the polycondensation reaction, the molds were sealed and heated at 80°C for 6 h.

Swelling degree

The swelling degree of the hydrogel samples (the ratio of the mass of water to the mass of polymers in the hydrogel sample) was determined according to the method described elsewhere [15].

Gel fraction content

The gel fraction content in the hydrogel samples was determined according to the method reported in ref. [16].

Rheological characterization of hydrogels

Rheological characteristics were measured using a stress-controlled rheometer (HAAKE MARS iQ). A parallel-plate geometry with a diameter of 25 mm was used, and the gap between the plates was adjusted to the sample height. The initial normal force in all measurements was 0.40 N. All rheological measurements were performed at 20°C. A frequency sweep was carried out at τ_0=150 Pa (determined within the LVER during the preliminary amplitude sweep) and f=0.1–15 Hz. The raw stress–strain signals were analyzed using HAAKE RheoWin 4 software. Stress–strain data affected by cohesive gel failure or slip at the gel–plate interface (adhesive failure) was excluded from the analysis.

Young's modulus was calculated according to the following equation:

$$E=2\cdot G'\cdot(1+\nu),$$

where G' is the storage modulus at a frequency of 1.01 Hz; and ν is Poisson's ratio; ν=0.5 was assumed (the case of ideal incompressibility for simplified calculations).

Collagen release

Collagen release from the hydrogel samples into water and model exudate was studied at 36°C as follows. Hydrogel samples (thickness 0.4 mm, diameter

Table 1

Synthesis conditions and characteristics of the copolymers*

No.	Concentration, %				Polymer yield, %	Carboxyl group content, mol.%
	AAm	AAc	MBAA	TGA		
1	14.94	4.85	0.16	0.44	88.05	15.7
2	10.02	9.84	0.15	0.45	79.20	37.2
3	14.49	4.77	0.15	0.29	90.20	9.8
4	14.63	4.82	0.15	0.19	92.50	9.6

Note: * – all syntheses were carried out at a structuring agent concentration of C_{PSA}=0.21% and a temperature of 50°C. The synthesis time was 4 h.

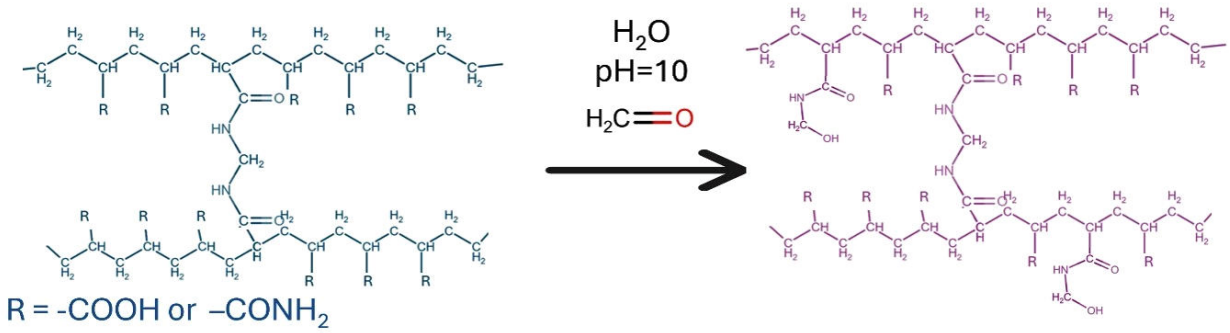


Fig. 2. Scheme of preparation of the structuring agent

2.25 cm) containing different amounts of collagen were placed in a tenfold mass excess of solvent. At predetermined time intervals, aliquots of the solution were taken, and the absorbance (D) was measured at λ=276.1 nm. The collagen concentration in solution was quantified using the corresponding calibration equations: C_w=(D−0.0052)/1.3775 for water and C_{ex}=(D+0.0066)/1.3838 for exudate. Based on these values, the amount of collagen remaining in the hydrogel was calculated.

Cytotoxicity testing

Cytotoxicity was evaluated according to the procedure described in ref. [16].

Fourier-transform infrared spectroscopy

Fourier-transform infrared (FTIR) spectra were recorded using a Spectrum Two spectrometer (PerkinElmer, USA) equipped with a universal attenuated total reflectance accessory with a diamond crystal. Spectra (16 scans per spectrum) were collected in the range of 4000−400 cm^{-1} with a resolution of 0.4 cm^{-1}. PerkinElmer Spectrum 10 software was used for spectral visualization.

Statistical analysis

Experiments were performed at least triplicate, and the data are presented as mean±standard deviation (SD). Mean values were compared using Student's t-test for independent samples. Differences were considered statistically significant at $P<0.05$.

Results and discussion

Collagen is the major structural protein of the body that forms a specific "framework" of connective tissues, including skin, bone, cartilage, tendons, and internal organs, thereby providing their strength, resilience, and elasticity. Owing to its unique biological properties, collagen is widely investigated as a component of hydrogels for biomedical applications, in particular for the development of wound dressings and soft-tissue regeneration systems, as evidenced by a considerable number of scientific publications. Collagen is a key component of the extracellular matrix of most soft tissues. Its interaction with cells plays an important role in wound healing and tissue remodeling, since collagen can support cell adhesion, proliferation, differentiation, and maintenance of cell phenotypes. In addition, collagen is characterized by biocompatibility, biodegradability, and low antigenicity, which makes it a promising material for tissue engineering and regenerative medicine [2,11].

Therefore, the development of hydrogels in which collagen can serve as an active component and be gradually released from the hydrogel matrix is both reasonable and promising.

The formation of the polymer network of the collagen−polyacrylamide hydrogel occurs owing to intermolecular interactions of the amide groups of the liquid-structured copolyacrylamide and collagen with the methylol groups of the polymeric structuring agent (the scheme is shown in Fig. 3).

Hydrogel samples were prepared according to the procedure described in the experimental section. The synthesis was carried out under conditions optimal for the formation of the polymer network of hydrogels based on the acrylic copolymers obtained by us. At a total concentration of prepolymers of 21%, a polymer-base-to-structuring-agent ratio of 1:1, and a temperature of 80°C, the collagen concentration varied from 0 to 7.5%. Table 2 presents the synthesis conditions and the characteristics of the polyacrylamide−collagen hydrogels determined during the study.

The gel fraction of the synthesized hydrogels is in the range of 45.8−65.7% (Table 2). The introduction of collagen leads to a relative decrease in gel fraction by 13−30% as the collagen concentration in the hydrogel increases from 3.5 to 7.3%. The gel fraction content is an important synthesis parameter because it enables controlled regulation of the mechanical properties of hydrogels for specific applications. Since the obtained polyacrylamide−collagen hydrogel is intended for use as a wound dressing for skin tissue regeneration, it should be resilient and elastic, fit well to the skin surface, and at the same time possess satisfactory mechanical properties. The gel fraction has a direct and significant effect on these properties: the higher the gel fraction (degree of crosslinking), the stronger, stiffer, and less elastic the material becomes.

This is since a higher gel fraction corresponds to a larger number of crosslinks (chemical or physical) in the three-dimensional polymer network. These crosslinks restrict the mobility of polymer chains, resulting in an increase in Young's modulus (stiffness) and the overall mechanical strength of the hydrogel. Therefore, the achieved gel-fraction values are satisfactory for ensuring a balance between elasticity and strength. The decrease in gel fraction upon addition of collagen may indicate that, under these synthesis conditions, collagen is not incorporated as a structural component of the hydrogel polymer network.

To evaluate Young's modulus, rheological studies of the hydrogel samples were performed according to the procedure described in the experimental section. Figure 4(a) shows the dependences of the storage modulus G' and the loss modulus G'' on the frequency of the applied deformation.

It was established that there is a relationship between the amount of collagen introduced into the composition and the elasticity of the sample, expressed

by the Young's modulus value at 1.01 Hz on the frequency sweep. The plot in Fig. 4(b) demonstrates a clear exponential dependence: increasing the collagen content in the gel-forming composition results in a sharp decrease in the sample elasticity parameter.

This may be explained by the fact that most spatial junctions in the hydrogel polymer network are formed by the copolymer of acrylamide, acrylic acid, and *N,N'*-methylenebisacrylamide together with the structuring agent obtained on its basis. Although collagen contains similar functional groups, its reactivity is not as high as that of the copolymer under these conditions, because the copolymer contains an NH_2 group in each repeating unit. As a result, side reactions that are unfavorable for the formation of the polymer network may occur, leading to a smaller number of elastically active bridges. This, in turn, reduces the effective crosslink density and increases

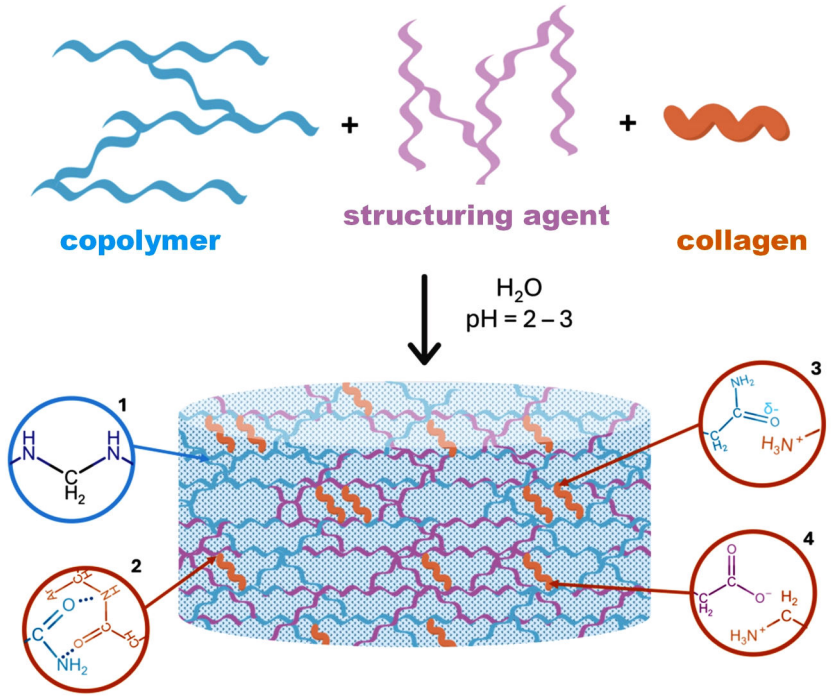


Fig. 3. Schematic representation of a combined hydrogel system based on polyacrylamide and collagen. The network architecture is stabilized through: (1) interchain covalent crosslinks between the copolymer and the crosslinker; (2) hydrogen bonding between acrylamide units and the collagen peptide backbone; (3) ion–dipole interactions between acrylamide units and amino groups of collagen residues; (4) ionic interactions between carboxyl groups of acrylic acid units (and distant amino acid groups of collagen) and amino groups of collagen residues

Table 2

Synthesis conditions and characteristics of the polyacrylamide–collagen hydrogel

No.	Component concentrations in the gel-forming mixture, %			Total concentration of gel-forming polymers, %	Characteristics of the polyacrylamide–collagen hydrogel				
	Structuring agent	Copolymer	Collagen		Gel fraction, %	Maximum swelling in water, g water/g polymer		Maximum swelling in exudate, g exudate/g polymer	
						20°C	36°C	20°C	36°C
1	10.88	10.92	0.0	21.79	65.7±4.7	9.18±0.92	17.43±1.74	17.01±1.70	22.74±3.41
2	10.84	7.31	3.50	21.66	56.8±3.2	12.34±0.99	13.62±1.91	19.62±2.75	22.54±2.25
3	10.56	5.37	5.73	21.65	55.3±3.5	9.61±1.15	12.77±1.53	19.77±1.58	25.57±3.07
4	10.68	4.38	6.52	21.58	54.8±2.8	8.03±0.64	12.95±1.04	11.36±0.79	17.24±1.55
5	10.79	3.41	7.31	21.51	45.8±2.5	8.08±0.73	15.04±1.80	15.88±2.38	23.05±3.27

the mesh size of the polymer network, making the hydrogel looser. One should also not exclude the possibility that, in a hydrogel containing 78% water, collagen is sufficiently solubilized so as not to exert any positive effect on the elasticity of the hydrogels [17]. Summarizing the Young's modulus results, it can be stated that the obtained polyacrylamide–collagen hydrogels can be applied for soft-tissue regeneration, since their Young's modulus is within 1–7 kPa, which meets the requirements for hydrogels of this purpose [18].

The swelling of the polyacrylamide–collagen hydrogels was studied in water and in model exudate at 20°C and 36°C. The obtained results are shown in Fig. 5(a–d), while the values of maximum swelling are presented in Table 2.

The obtained results demonstrate an increase in the swelling degree of all collagen-containing hydrogel samples both at 20°C and 36°C. It should be noted that the swelling degree in exudate is higher for all obtained collagen-containing hydrogels than that in water at all temperatures. This can be explained by the effect of the pH of the swelling medium (pH=5.5 for water and pH=7.8 for exudate), since it is known that the pH of the solution strongly affects the swelling of collagen-containing hydrogels because collagen is a polyampholyte, i.e., a compound containing both acidic (carboxyl) and basic (amino) groups. With increasing pH, carboxyl groups become deprotonated ($-COO^-$), and the molecule acquires a negative charge. This generates repulsive forces and promotes hydrogel swelling.

In addition, a dependence on swelling on collagen content is observed both in water and in exudate at all temperatures. The sample containing 3.53% collagen exhibits the highest swelling values over the entire time range of the study, whereas a further increase in collagen concentration leads to a decrease in swelling. This could be explained by assuming that an increase in collagen concentration increases the number of crosslinks and forms a denser hydrogel with a smaller free volume for swelling. However, this interpretation contradicts the rheological results, which clearly show that Young's modulus decreases with increasing collagen concentration.

The explanation is likely that the introduction of collagen into the hydrogel does not form a homogeneous elastic network, because collagen forms fibrils/fibers, and therefore the collagen-containing hydrogel exhibits nonlinear mechanics (soft at small deformations and strain-stiffening at larger deformations). Therefore, an increase in collagen content does not lead to an increase in Young's modules. The introduction of collagen into the three-dimensional hydrogel network decreases the density of the effective network; in addition, ungrafted collagen fibrils are poorly connected with the main network, i.e., they «float» in the hydrogel matrix and therefore do not bear the load at small deformations, making the hydrogel material softer.

The remaining question is why swelling first increases with increasing collagen content and then decreases. At low and moderate collagen contents, swelling increases because of the hydrophilic properties of collagen, which contains a large number of –OH, –COOH, and $-NH_2$ groups; upon swelling, osmotic

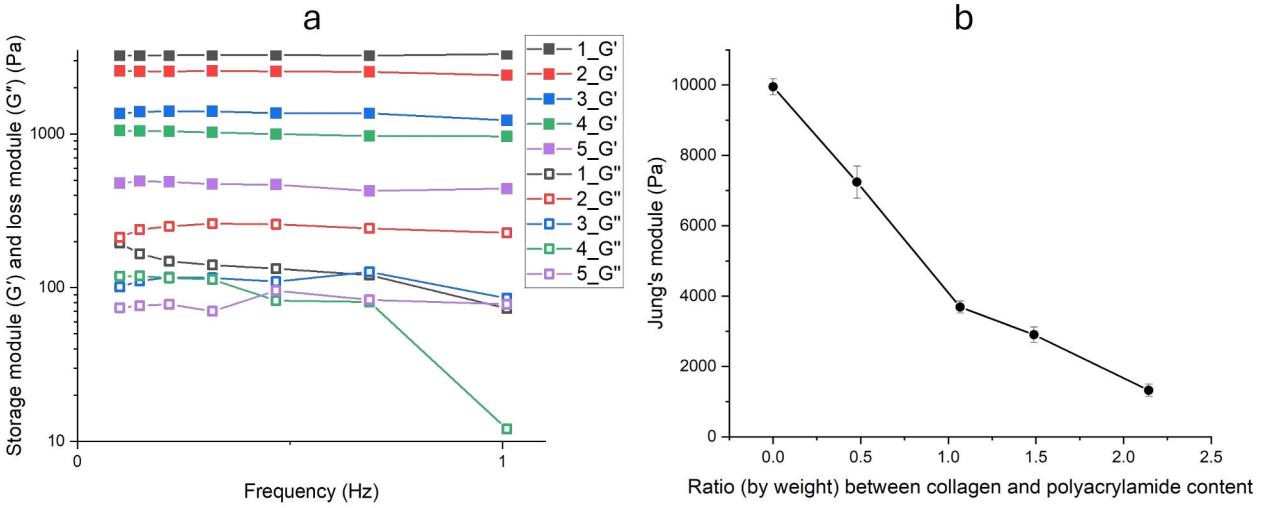


Fig. 4. (a) – Storage modulus (G') and loss modulus (G'') of the hydrogels during a frequency sweep with frequency gradually increased from 0.1 to 1.0 Hz; (b) – dependence of the calculated Young's modulus on the mass ratio between collagen and polyacrylamide in the hydrogel composition

pressure increases and, accordingly, water uptake and the swelling degree increase despite the decrease in Young's modulus. With a further increase in collagen content, dense fibrillar domains are formed, which leads to hydrogen-bond formation and hydrophobic interactions between fibrils, and the network becomes topologically stiffer. This causes elastic resistance to swelling, and the swelling degree decreases. The topological increase in stiffness refers to an increase in constraints on network rearrangement caused by fibrillar interactions and physical crosslinks, which limit swelling.

Since the developed material is positioned as a promising agent for the local treatment of wound surfaces, a key aspect of its functional efficiency is the kinetics of collagen release. Collagen is the main structural protein of the extracellular matrix, and its timely, controlled delivery to the damaged area is critically important for all stages of the wound-healing process. At the initial stages, the released protein promotes hemostasis and the migration of inflammatory cells. Subsequently, it acts as a matrix (scaffold) for fibroblast proliferation and angiogenesis, thereby stimulating granulation tissue formation and accelerating epithelialization. Prolonged and uniform collagen release from the material matrix makes it possible to maintain an optimal therapeutic protein concentration in the wound and to prevent its premature enzymatic degradation under the action of collagenases of bacterial or tissue origin. Thus, investigation of the collagen-release profile is critically important for predicting the regenerative potential of the created material *in vivo*.

The kinetics of collagen release from the polyacrylamide–collagen hydrogels was studied at 36^{0}C in water and in model exudate. The obtained kinetic curves for all samples have a similar character and indicate a two-stage mechanism of collagen release.

At the initial stage of the experiment, rapid collagen release is observed, which is due to the diffusion of weakly bound or solubilized protein molecules localized in the near-surface layers of the hydrogel matrix. After this stage is completed, the process passes into a slower diffusion-controlled regime

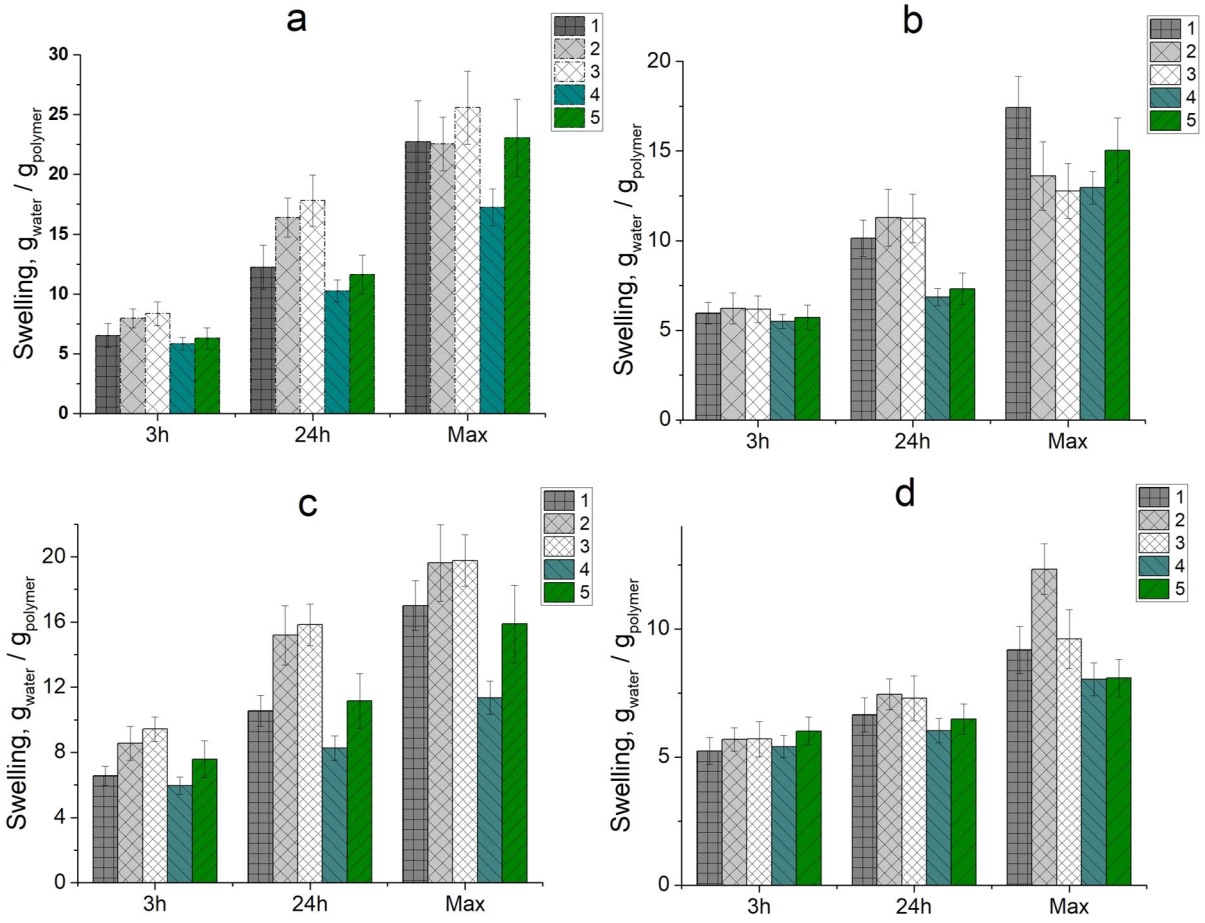


Fig. 5. Dependence of the swelling degree of hydrogel samples on time: (a) in model exudate at 36^{0}C; (b) in water at 36^{0}C; (c) in model exudate at 20^{0}C; (d) in water at 20^{0}C

characterized by a gradual decrease in release rate and an approach to a plateau (Fig. 6(a,b)).

Comparison of the results obtained in different media revealed certain differences in the character and degree of collagen release. In water, the release process proceeds faster, and the total amount of collagen released after 24 h reaches about 90% of its initial content.

This is also confirmed by FTIR analysis: collagen peaks are absent in the spectrum of the gel fraction of the polyacrylamide–collagen hydrogels (Fig. 7). A more detailed interpretation of the FTIR spectra supports this conclusion. The spectrum of collagen is characterized by the typical protein bands, namely amide A in the region of 3300–3400 cm^{-1} (N–H and O–H stretching vibrations), amide I at about 1630–1650 cm^{-1} (mainly C=O stretching of the peptide bond), amide II at about 1540–1555 cm^{-1} (N–H bending combined with C–N stretching), and amide III in the region of 1230–1245 cm^{-1}, which is also sensitive to the collagen secondary structure. In the spectra of the gel fraction of the polyacrylamide–collagen hydrogels, these characteristic collagen bands are absent or do not appear as distinct collagen-specific features. In particular, no separate amide I and amide II bands attributable to collagen are observed, which indicates that collagen is not present in the isolated gel fraction in an amount sufficient for spectral detection. This result is consistent with the release experiments and confirms that collagen is not covalently incorporated into the permanent polymer network but is retained in the hydrogel mainly through noncovalent interactions and can therefore be removed during extraction or released into the medium.

In model exudate, the release rate is lower, and the maximum release degree does not exceed 75%. The lower intensity of collagen release in exudate may be associated with the higher ionic strength of the medium and the presence of electrolytes, which screen the charged groups of collagens and promote the formation of additional physical interactions between the protein and the polymer matrix. This leads to a decrease in the effective diffusion coefficient of collagen and its partial retention within the hydrogel volume.

The results obtained may indicate the following. In the acidic medium used for hydrogel synthesis (pH=2), the reaction of N-hydroxymethylacrylamide proceeds predominantly with polyacrylamide. This can be explained by several mutually reinforcing factors. First, in an acidic medium the main reactive groups, namely the amino groups of lysine, become protonated to $-NH_3^+$, and their nucleophilicity decreases sharply, so that they lose the ability to attack the methylol fragment of N-hydroxymethylacrylamide. Thus, in an acidic medium collagen becomes less reactive because of amino-group passivation. In addition, polyacrylamide is more mobile in such a solution, contains many amid groups, and these are accessible along the entire chain and capable of condensation in an acidic medium. Therefore, the hydrogel synthesized under these conditions contains collagen that is bound by noncovalent interactions, which explains the partial decrease in mechanical properties while still allowing collagen release.

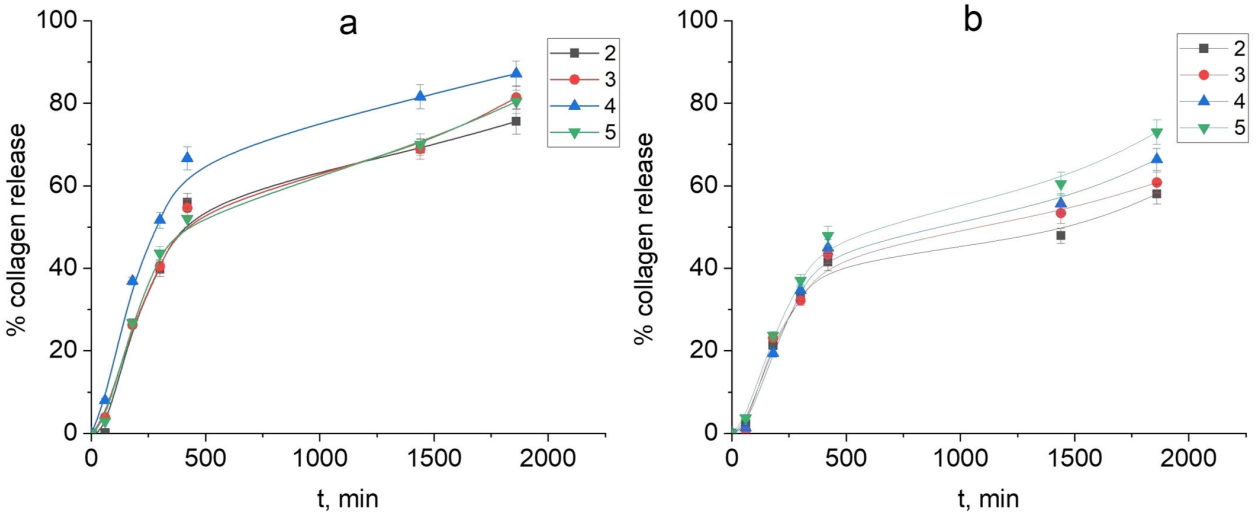


Fig. 6. (a) – Dependence of collagen release into water from the polyacrylamide–collagen hydrogel (sample numbers correspond to those given in Table 2); (b) – dependence of collagen release into model exudate from the polyacrylamide–collagen hydrogel (sample numbers correspond to those given in Table 2)

For the use of the obtained polyacrylamide–collagen hydrogels as wound dressings, it is important to investigate their cytotoxicity. However, after synthesis the hydrogel contains a catalyst and may contain residues of unreacted starting substances. The results of the cytotoxicity study of hydrogel on living cells are presented in Table 3.

It was established that the hydrogel sample immediately after synthesis (sample 2 from Table 2) exhibits a bull spermatozoa survival time of 68±4.20 h. Considering that the control value was 114.0±5.20 h, a decrease in spermatozoa survival time of 59% is observed. According to the accepted in vitro cytotoxicity standard ISO 10993-5, this sample exhibits toxic properties, since «a reduction in cell viability by more than 30% is considered a cytotoxic effect.»

Therefore, studies were carried out on the purification of the obtained hydrogels. It was found that the samples were washed in an 88% isopropyl alcohol solution according to the procedure described in the experimental section. During washing, partial collapse of the hydrogels occurred with partial water loss. Therefore, before cytotoxicity testing, they were resaturated with distilled water to the initial swelling degree. At the same time, residual alcohol was also removed from the hydrogels. The results presented in the table for different durations of contact with the alcohol solution (2, 4, and 6 h) show that cell survival tended to increase, and after 6 h, it reached 94.7% of the control value. This indicates that after this purification procedure for 6 h, the hydrogels can be considered noncytotoxic and safe for biomedical applications.

Conclusions

A series of hybrid polyacrylamide–collagen hydrogels based on copolymers of acrylamide, acrylic

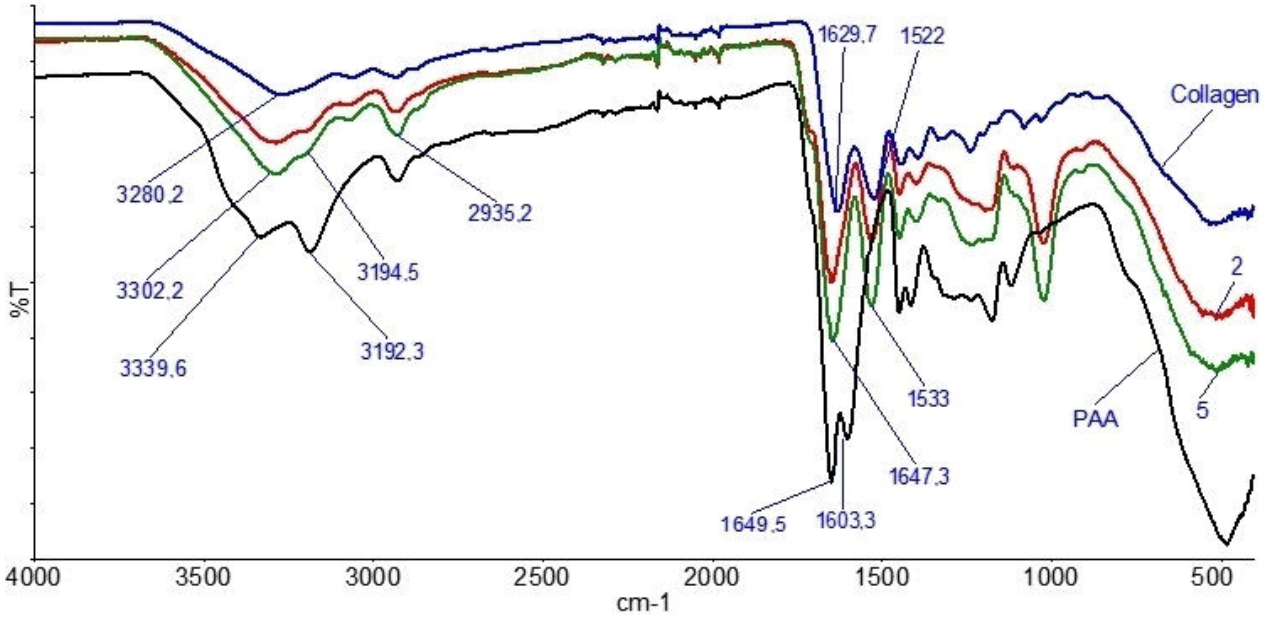


Fig. 7. FTIR spectra of polyacrylamide, collagen, and the polymer gel fraction of the polyacrylamide–collagen hydrogel samples (samples 2 and 5 from Table 2) in the range of 4000–400 cm^{-1}

Table 3

Survival of bull spermatozoa under the action of the gel fraction of polyacrylamide–collagen hydrogels, n=4

No.	Sample type	Survival, h	
		M±m	CV
1	Control	114.0±5.20	9.1
2.0	dose 10 mg/mL, washing time 0 h	68.0±4.20	10.2
2.2	dose 10 mg/mL, washing time 2 h	88.0±6.00	11.1
2.4	dose 10 mg/mL, washing time 4 h	99.0±6.00	10.7
2.6	dose 10 mg/mL, washing time 6 h	108.0±6.00	11.5

acid, and *N,N'*-methylenebisacrylamide was synthesized using a polymeric structuring agent that ensured the formation of a three-dimensional hydrogel network.

It was established that the introduction of collagen into the hydrogel matrix leads to a decrease in the gel fraction and the effective crosslink density, which is reflected in a reduction of Young's modulus and the formation of softer and more elastic materials suitable for soft-tissue regeneration applications.

It was shown that the dependence of the swelling degree of polyacrylamide–collagen hydrogels on collagen content is nonmonotonic and is determined by a combination of the hydrophilic properties of collagen, osmotic pressure, and topological constraints caused by the formation of fibrillar domains.

It was established that collagen is capable of being released from the hydrogel matrix, indicating that it is not incorporated into the polymer framework of the hydrogel through stable covalent bonds. Investigation of collagen release kinetics at 36^0C revealed a classical two-stage process with an initial rapid stage followed by a diffusion-controlled regime. It was found that about 90% of collagen is released from the hydrogel samples in water, whereas no more than 75% is released in model exudate, indicating partial retention of collagen within the hydrogel matrix.

The obtained results confirm the possibility of regulating the mechanical properties, swelling behavior, and collagen-release profile by varying the hydrogel composition, which is important for the further optimization of wound-dressing materials with prolonged biological activity.

The obtained material showed no cytotoxicity, which makes it promising for biomedical applications.

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СИНТЕЗ ТА ДОСЛІДЖЕННЯ ВЛАСТИВОСТЕЙ ГІБРИДНИХ КОЛАГЕН–ПОЛІАКРИЛАМІДНИХ ГІДРОГЕЛІВ

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Було синтезовано гібридні колаген–поліакриламідні гідрогелі та досліджено їхні властивості з використанням кополімерів акриламіду, акрилової кислоти та N,N'-метиленбісакриламіду в присутності полімерного структурувального агента. Тривимірну полімерну сітку формували шляхом поліконденсації в кислому середовищі за підвищеної температури. Вивчено вплив вмісту колагену на структуру, ступінь зшивання, механічні властивості, поведінку при набряканні та кінетику вивільнення колагену з гідрогелів. Показано, що введення колагену до гідрогелевої матриці приводить до зменшення гель-фракції та модуля Юнга внаслідок зниження ефективної густини зшивання та формування неоднорідної фібрилярної структури. Виявлено немонотонну залежність ступеня набрякання від концентрації колагену, що зумовлено конкуренцією між осмотичними ефектами та топологічними обмеженнями полімерної сітки. Дослідження вивільнення колагену за фізіологічної температури (36^{0}C) показало двостадійний процес і виражену залежність від середовища: у воді вивільняється до 90% колагену, тоді як у модельному ексудаті – до 75%. Отримані результати демонструють перспективність колаген–поліакриламідних гідрогелів як основи для створення ранових покриттів із контрольованими механічними властивостями та пролонгованим вивільненням біологічно активних компонентів.

Ключові слова: поліакриламід, колаген, гідрогель, набрякання, кінетика вивільнення, ранові покриття.

SYNTHESIS AND STUDY OF THE PROPERTIES OF HYBRID COLLAGEN–POLYACRYLAMIDE HYDROGELS

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Hybrid collagen–polyacrylamide hydrogels were synthesized and their properties were investigated using copolymers of acrylamide, acrylic acid, and N,N'-methylenebisacrylamide in the presence of a polymeric structuring agent. The three-dimensional polymer network was formed by polycondensation in an acidic medium at elevated temperature. The effect of collagen content on the structure, crosslinking degree, mechanical properties, swelling behavior, and collagen release kinetics of the hydrogels was studied. The incorporation of collagen into the hydrogel matrix was shown to decrease the gel fraction and Young's modulus because of a reduced effective crosslink density and the formation of a heterogeneous fibrillar structure. A nonmonotonic dependence of the swelling degree on collagen concentration was observed, which was attributed to the competition between osmotic effects and topological constraints of the polymer network. Investigation of collagen release at physiological temperature (36^{0}C) revealed a two-stage process and a pronounced dependence on the release medium: up to 90% of collagen was released in water, whereas up to 75% was released in model exudate. The obtained results demonstrate the potential of collagen–polyacrylamide hydrogels as a basis for the development of wound dressings with controlled mechanical properties and prolonged release of biologically active components.

Keywords: polyacrylamide; collagen; hydrogel; swelling; release kinetics; wound dressings.

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